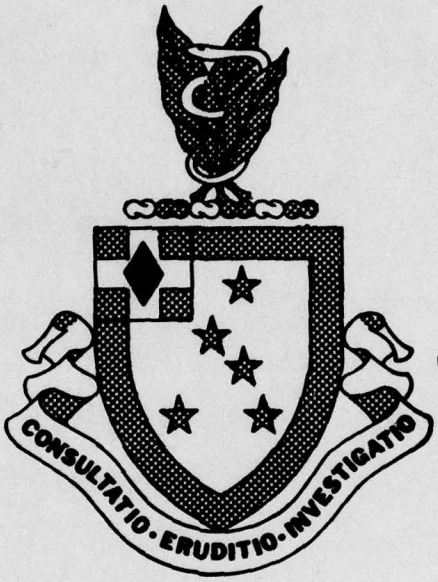
ARMED FORCES INSTITUTE OF PATHOLOGY



1 October 1976 - 30 September 1977

U.S. ARMY

MEDICAL RESEARCH AND DEVELOPMENT REPORT

RCS MEDDH-288 (RI)

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In conducting the research described in this report, utilizing animals, the investigators adhered to the 'Guide for the Care and Use of Laboratory Animals,' as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences - National Research Council.

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U.S. ARMY RESEARCH AND DEVELOPMENT

TECHNICAL REPORT

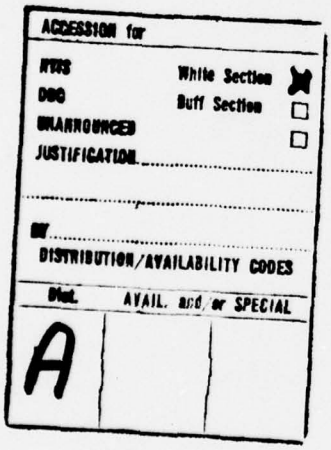
RCS-MEDDH-288(R1)

ARMED FORCES INSTITUTE OF PATHOLOGY

Washington, D. C. 20306

ANNUAL PROGRESS REPORT

1 OCTOBER 1976 - 30 SEPTEMBER 1977



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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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(U) Analysis of Cytotoxic Reactions Produced by MUST-Water Constituents

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23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Develop cytoassay for detection of MUST-water impurities through extracting quantitative morphologic descriptors from digital images of cells cultured in waters tested. Method should extend to general water testing. Implication: reduce need for animal toxicologic testing. Desired result: higher and more quantitative data rates: lower costs; simpler laboratory logistics. Application to MUST re-use water primary; extension to water testing where military presence is possible, probable, or actual within applicability. Military-industrial effluent cytoassay an important consideration.

24 (U) Purchase upgrade for minicomputer hardware and software. Upgrade image digitizing light microscope subsystem. Design and fabricate (D&F) electronics for interfacing/control of scanner to/by minicomputer. Generate application specific programs. Standardize culture and staining procedures. Run feasibility experiments with raw water and water with known toxic additives; technical problems; standardize culture in presence of toxicants/raw water; theoretically develop and implement quantitative estimator of staining variability in contrasts and intensity; pre-analysis validation of image data; extract cell image descriptors of cytopathologic value which sensitive to exposure time and toxicant concentration; production of adequate experimental throughout on modest minicomputer system. Decelerating factors; procurement delays and defects in new equipment and software. Accelerating factor: addition of 1 man-year/year of professional effort, starting FY75.

25 (U) 7610-7709. Electronic interfacing and testing of equipment added to upgrade scanning microdensitometry system completed. Program subsystem for interactive acquisition of digital test cell images completed. This subsystem allows acquisition of test cell images without prior standardization of cell preparatory techniques.

*Available to contractors upon originator's approval

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY (DD Form 1498) (Page 2)

Title: (U) Analysis of Cytotoxic Reactions Produced by MUST-Water Constituents

Project No. 3E762720A835

Cell image data is recorded in format appropriate for input to analytic subsystem software which will perform image measurements and statistical analysis. Development of image analysis subsystem in progress.

ANNUAL PROGRESS REPORT

TITLE PAGE

Project No. 3E762720A835 Title: Analysis of Cytotoxic Reactions Produced by MUST-Water Constituents

Task No. 00

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Armed Forces Institute of Pathology
Washington, D. C. 20306

Name of Department: Cellular Pathology

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Professional Authors: Joseph A. Boccia, LTC, MC, USA
Gunter F. Bahr, M.D.

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BODY OF REPORT

Project No. 3E762720A835 Title: Analysis of Cytotoxic Reactions Produced by MUST-Water Constituents

Task No. 00

A computer assisted scanning light microscope for obtaining digital cytologic images has been completed and is now in full operation at the Department of Cellular Pathology of the AFIP. This scanner is a subsystem of a full system for the analysis of cytotoxic reactions to noxious agents. The complete system is under development for testing the feasibility and sensitivity of a cytologic assay for non-specific detection of water impurities.

The scanner (subsystem) is currently applied to the acquisition of cell images from experiments with Mouse-L cells exposed to varying concentrations of model toxicant in cell culture. Digital images of such cells are obtained by using the scanner, which ultimately enables the recording of such cells on nine track magnetic tape. These images will subsequently be analysed for morphologic differences among cells exposed to varying concentrations and for different amounts of time in culture.

The Mouse-L cells being scanned at this time have been exposed to dinitrophenylhydrazine (DNPH) for periods ranging from one to four days. After removal from culture and fixation, the cells are stained with Feulgen and Napthol Yellow and scanned. Included among the various cells are those which have been exposed to culture media without toxicant. These serve as controls for comparison with those exposed to toxicant.

Laboratory technical personnel, having backgrounds in histotechnology and cytotechnology, have been trained in the operation of the scanning subsystem. Such individuals have been able to learn scanner usage in approximately ten training days, during each of which instruction is given for a period of about three hours. After such training, the individual is capable of operating the scanner, assisted only by occasional instruction from the Chief Operator. The latter individual is specially trained in greater depth for trouble shooting and system operations not absolutely required in the repertoire of those actually scanning cells.

Further experiments are planned, including the testing of different water samples and reducing the concentration of model toxicant against which cellular morphologic reactions are assessed. At present, the principal production activity is the scanning of cells exposed to DNPH, and this will be followed by the scanning of those exposed to RO Permeate concentrate. Cells from time-concentration experiments with the latter are already available in fixed and stained form.

Title: Analysis of Cytotoxic Reactions Produced by MUST-Water Constituents

The principal development activity still required is the implementation of computer software for the making of morphologic measurements and certain control programs for the performance of the statistics required in analysis of data. The detailed statistical computations will be carried out using a scientific software package supplied through the user's group of the minicomputer's manufacturer. This package includes necessary statistical and mathematical programs in the form of subroutines. The control programs alluded to are for selection and sequencing statistical subroutines which comprise the particular analysis, the input of data for analysis from magnetic tape, and the printout of results. While development of this software is taking place, cells are being scanned and the images along with pertinent associated data are stored in a laboratory standard format on nine track magnetic tape. When the analytic portions of the system software are available, the cellular images currently being acquired and stored on tape will be appropriately analysed.

THE SCANNING LIGHT MICROSCOPE SUBSYSTEM (SCANNER)

The scanning subsystem consists of three major components: 1) a Zeiss universal microscope equipped with 0.5 micron motorized stepping stage, 2) a microdensitometer and associated analogue-to-digital (A/D) conversion electronics, and 3) an Interdata Model 70 (Perkin Elmer Data Systems), 16-bit minicomputer equipped with magnetic disc and tape. The magnetic tape used is nine track, industry (IBM) compatible and total disc capacity on two drives is 20 megabytes (20 mission characters). One electrostatic line printer/plotter is available and two teletype terminals are used for interactive communication with the system. Further details on the anatomy of this system, which has been developed specifically for the purpose of this investigation, are not presented here. Such detail may be found in a previous AFIP report (1).

The operation of scanning selected cells from cultures in which they have been exposed to model toxicants begins by placing the glass microscope slide on the stepping stage of the scanning microscope and orienting the scanner's eyepiece crosshairs on anyone of four marks impressed on the slide by an objective containing an industrial diamond. A mechanical device for making such impressions standard in microscope slides has been designed and fabricated in this laboratory.

Once oriented on a mark and having informed the system of the mark chosen by teletypewriter, the operator may move about the slide while observing for cells to scan. Such movement may be accomplished in a variety of ways which involve either a keyboard command on the teletypewriter or manipulation of a joystick to which the stepping stage responds. In any case, the system keeps constant track of location with respect to the selected mark. Facilities are included which allow the random choice of cells, thus avoiding bias in the choice of those for scanning. Since a clone is being used as a test species, all cells are presumed equally valid candidates for assessment. Therefore, the selections

made are from an uniform, bivariate distribution over the slide area containing cells. Suitable random coordinate generating software is included in the scanning subsystem to allow this method of cell selection.

Random selection need not be utilized. Any form of directed search, by joystick or teletypewriter command, may be utilized. Further, even in the random selection of cells, one exception to random selection is made. Cells in observable mitosis are not sampled. It is, therefore, more accurate to say that, for these studies, cells not in observable mitosis are randomly selected for scanning.

Once a cell is chosen for scanning, its location is recorded along with its digital image. In fact, a large number of associated data items are recorded with the image. In addition to the cell's location on the slide, such items as the scanning date, the operator's name, the slide identifier, the stains used, the cell's identifier, the cell type, the optics used (multiple items), toxicant exposure and time of exposure are recorded. Over 60 associated data items are currently being recorded with each cell's digital image. Software for defining new data items and removing old ones is available as part of the utility subsystem.

Though a large number of data items is maintained, only those which change from cell to cell are updated. The others are simply recorded with each image. Any item may be updated, but the ones which change from cell to cell must be. The system forces such mandatory updates from cell to cell in one of two ways: 1) The operator is not allowed to proceed with the next cell unless the updated data are entered, or 2) the system automatically performs the update without any requirement for input from the operator. Which method is used depends upon the specific item requiring update. If the system can determine the updated value for a particular item, then the update is automatic. Otherwise, the operator must input the new information. After the actual scan of any cell is completed, all data items requiring update for the next cell are erased from the system memory. Scanning and recording of the next cell is not permitted in the presence of incomplete data, so the erased items must be filled. At various points in the processing of the next cell selected, the system will either update an item automatically or the operator will be prompted to input the needed data.

In an entirely analogous manner, information which changes from slide to slide (e.g., slide identifier, reference mark number, etc.) is erased when orienting on a mark with new slides. This forces update of the level of information, since scanning in the presence of incomplete data of any level is not permitted. Similarly, information which changes from scanning session to session (e.g., date and operator's name) is purged when the system is logged out after completion of a scanning shift. Update of this level of information is also forced.

Thus, a large amount of information is maintained and recorded with each digital image. However, the operator is concerned with maintenance of only a small portion when passing among the cells. Errors and time are reduced, while information useful or necessary for analysis is consistently recorded.

In addition to date recorded in alphanumeric format, the system requires certain graphic items best considered tracings. This data takes the form of curves or points, drawn on and about the scene with joystick and crosshairs, consisting of the selected cell and its immediate slide neighborhood. Absolutely required is a closed loop curve, called a scanning perimeter, around the selected cell which separates it from all neighbors. Additional graphic annotation of cellular morphology is both permissible and optional. Currently, cellular and nuclear borders are traced by the operator. The various graphic annotations are used during physical scanning and subsequent analysis of the cell. The number and nature of these tracings are alterable by using software facilities built into the system. The operator is prompted to complete every required tracing for the cells in question. Any attempt to scan in the presence of incomplete graphic data is rejected and, upon successful completion of any cell scan, the graphic data is erased from system memory - provided it is also successfully recorded on magnetic tape. Thus the system prevents accidental duplication of the same graphic data for different cells. If, at the operator's option, the cellular data is not recorded on magnetic tape, then no associated data is purged and the cell may be repeatedly scanned without repetitious completion of the associated data.

For every cell, the associated and scanning data are first recorded on magnetic disc. This enables computer operations which make use of direct data access, as distinct from the sequential access typical of magnetic tape operations. When both associated and scanning data are completed on disc, the scanning subsystem transfers it, in appropriate format for future analysis, to magnetic tape. This process assures that all data required to 1) read the magnetic tape's information back to disc, 2) analyse the cellular image, and 3) display results appropriately identified as belonging to a particular cell is recorded. Similarly assured is an image data format which facilitates computerized examination of the cell picture.

While the cell is being scanned, the data obtained is placed on magnetic disc in a manner which breaks the picture into rectangular sections, each of which consists of a partial digital image having dimensions 16 by 16 pixels. The image is subsequently recorded on tape in sequential, section oriented format. When read back onto disc at analysis time, the digital image will be transferred in this sectional form and require no additional sort to achieve this arrangement. Thus, though physical scanning provides image data in scan line order, the system sorts each image point value into the proper image section during scanning. Once in this form, the image is maintained as such on both disc and tape. The data is transferred between tape and disc, when necessary, but no rearrangement is required. Such reformating would require additional time on any occasion a cell's image is transferred between disc and tape. Since such transfers are performed at analysis time, unnecessary repetitions of scan line to section sort are avoided by performing this operation once at time of scan. The scanning time per cell is increased, but the price is paid only once. Subsequent utilization of any digital image requires no sorting of this type.

If it is desired to scan a particular cell(s) using filtered light in different passbands, up to five spectrally distinct image versions of the same cell may be recorded with the same set of associated data items. This capability permits assessment of various staining techniques at different wave lengths of visible transillumination. For each spectrally different version required, the physical scan must be repeated. It is not, however, necessary to repeat either the required tracing or enter any updates to alphanumeric data. If multiple images are desired, the system can be informed and the operator is both prompted and required to scan the appropriate number of times, changing filters appropriately (as prompted) prior to each scan.

CURRENT DATA ACQUISITION

The scanning subsystem is dedicated 100% to the acquisition of data testing the feasibility of a cytotoxicity assay for water purity. At this writing, the scanning of Mouse-L cells (clone 939) exposed to varying concentrations of Dinitrophenylhydrazine (DNPH) is being performed. The cells are exposed to toxicant on 1 x 2 cm glass coverslips for differing amounts of time in culture. When a coverslip containing test cells is removed from culture, it is immediately fixed in ethyl alcohol and subsequently stained. For current DNPH experiments, the Feulgen nuclear reaction is being used with a Napthol Yellow counterstain.

The Feulgen staining reaction is used for the stoichiometric coloration of DNA and renders the cell nuclei a magenta color which absorbs light at a peak wave length of approximately 560 nanometers. Similarly, Napthol Yellow stains protein and absorbs at a peak of approximately 435 nanometers. Although the scanner permits the digitizing of multiple image versions for each cell, only a single image at 560 nanometers is being recorded for initial experiments.

The grey scale being used is derived from optical density. For each pixel (picture element) in the digital cell image, the datum recorded is 100 x OD where OD is the optical density of the point measured. Such optical densities range, in practice, between 0.00 and 1.80 so the data recorded lie in the range 0 to 180. For Feulgen and Napthol Yellow stained Mouse-L cells, blank slide and coverslip are calibrated (near every cell scanned) to 0 grey value. Under these calibration conditions, cytoplasm measures in the range of 3 - 12 grey value units and nucleoplasm varies between approximately 23 and 50 grey value units. Thus, the Feulgen and Napthol Yellow stains give sufficient nucleocytoplasmic contrast for utility in subsequently intended image analysis - which will require approximately 5 grey value units of contrast to assure distinction between two cellular regions on the basis of grey level. Generally, grey values are measurable to +/- 2 units reproducible. A contrast of 5 units, therefore, gives sufficient margin for variations in staining. In actual experience, the Feulgen and Napthol Yellow staining reactions give nucleocytoplasmic contrast of 10 or more units.

Cells currently being scanned are exposed to DNPH for 1, 2, 3, or 4 days against concentrations of 0.0, 0.10, 0.32, 1.00, 3.20 and 10.00 milligrams per liter. Thus, for each duration of exposure in culture and each concentration

there is at least one coverslip (frequently replicates) containing a population of cells similarly exposed. For the experiment described, there are 24 such populations from which 25 cells are randomly selected for image capture. Thus, a total of 600 cell images must be captured for the one experiment. Similar experiments using other model toxicants are planned. Further, if cytologic intoxication can be detected by quantitatively measurable morphologic alterations, experiments with DNPH in lower concentrations and for shorter culture times will be conducted. The objective of such reduction of exposure time and toxicant concentration is the determination of practical sensitivity limits in the sense of both duration and intensity of exposure to noxious agents. In all cases, acute toxicity assessment is the goal.

As cells are scanned, the corresponding images are recorded on nine track, industry compatible magnetic tape. The format utilized has been developed at this laboratory for the data banking of cell images. The data are arranged in such a manner that pertinent associated information is recorded with each image. In particular, the cell identifier, the time of exposure in culture, the concentration and nature of toxicant(s), the staining reactions employed, the light microscopic optical parameters regarding magnification and resolution, and the spatial resolution at which the physical scanning of the cell is actually carried out are among the data recorded. In all, over 60 associated items are recorded with each cell image. The scanning subsystem permits expansion of these as required.

Certain graphic annotations are interactively generated and stored among the associated data for each cell. Specifically, three tracings are performed by the scan operator in the course of scan. A perimeter is traced about the cell to separate it from its neighbors. Only the interior of such a perimeter is included in the acquired digital cell image. Therefore, each cell image captured is cleaned (edited) to free it from unwanted detail. Also traced are the cellular and nuclear borders. These tracings are recorded for use during analysis of the image. Such tracings are made using a joystick and the microscope's eyepiece crosshairs. By joystick manipulation the operator (via motorized stage) can track the crosshair intersection through any desired path. The trajectory of this path is memorized and recorded with the rest of the image data.

Not all data changes from cell to cell during scanning for a given experiment. Only such data items which require update from cell to cell are actually altered between scans. This update, where necessary, is carried out by either the operator or automatically by the system - depending upon the nature of the data item. Generally, if the system can know the value for an updatable item, then the operator's input is not required and the update is performed automatically. An example of this is cell location on the slide. The scanner always maintains the co-ordinates of any current location by continuously altering appropriate indicators during movement of the slide. It is, therefore, not necessary for the operator to supply (or even know) such information.

The size of cell being scanned,the data format used for recording on magnetic tape and the amount of associated data recorded for each image are such

that approximately 750 cells may be recorded on each 2400 foot reel of magnetic tape. Assuming continued experiments of approximately 25 different cell populations (by toxicant concentration and duration of exposure in culture) and scanning of 25-30 cells per population (coverslip), one reel of magnetic tape shall contain the cells from each complete experiment.

The acquisition of cell images occupies 4 hours of each laboratory working day. During the remaining 4 hours of the normal duty shift and any additional time required, the minicomputer system is used for the development of programs performing the extraction of quantitative morphologic measurements (features) from the cell images which have been recorded. When these programs have been prepared (preparation now in progress), they will be run using the mass of scanned cells as input. Statistical analysis of resulting feature data will be based upon usage of a scientific subroutine library obtained from the user's group of the minicomputer manufacturer. When such software is ready, no scanning will be performed on days it is run. Such analytic runs on previously captured images will occupy 100% of the minicomputer resources until successful run completion.

Additional tests will be performed using RO Permeate concentrate in place of DNPH. Preliminary qualitative results by both light and scanning electron microscopy indicate that RO Permeate causes morphologic alterations in Mouse-L cells which are at least as severe as DNPH effects.

TECHNICAL PROBLEMS

During the reporting period, various problems have arisen. Some have been solved and others are in the process of being solved. None of the technical considerations discussed below interfere with scanning of cells. Principally effected are scanning rate and data security.

Initially, an attempt to calibrate the microdensitometer at a single position over clear microscope slide was made. At such a position, the microdensitometer is adjusted to register 100% transmission, which is a grey value of 0. It was subsequently learned that an apparently clear slide can vary between 93% and 100% of the transmission at the selected calibration position. Such variation in slide background is unacceptable, so the microdensitometer has to be calibrated in the neighborhood of every cell selected for scanning. This calibration is performed in a clear slide area several microns from the cell edge. The necessity to perform this adjustment results in a slowing of cell acquisition rate. However, too wide a variation in light absorbence contributed by the glass slide and coverslip will complicate cell to cell comparisons among those from the same slide - not to mention cells from different slides. Calibration is, therefore, performed near each selected cell with any required filters in the light path. The scanning software provides support for such functions as 1) recording the calibration location, 2) allowing for trimming the settings between scans for spectrally distinct images and 3) calibration at the same slide position after remounting the slide. This software support compensates partially for the additional time required to calibrate next to every selected cell.

Title: Analysis of Cytotoxic Reactions Produced by MUST-Water Constituents

Another problem which has presented itself concerns the use of filters. It is convenient for the operator to remove filters from the light path when searching for cells or performing tracing annotation once a cell is selected. This allows visualization in white light. The interference filter used exerts a prismatic effect on the light beam, resulting in loss of calibration when re-inserted. This is corrected by assuring repositioning identical to that when calibration is performed. The problem has been solved by machining a metalic ring into which the round filter is fitted. This ring, in turn, can be positioned in only one way into the corresponding filter holder on the microscope. The filter holder is hinged and may be swung into the light path as required. The ring and filter fit snuggly and do not move during the procedure. Calibration is not lost by removing and replacing the filter.

Many of the Mouse-L cells found in the culture preparations are extremely dendritic or fusiform in shape. Performing a rectangular scan throughout a framing rectangle results in much motion over slide area that contains no cell. The subroutine (program) which performs scanning at present is already quite complex, since both the meander scanning pattern and proper sectional sorting of the scan data must be carried out in one pass over the cell. However, the physical scan can be shortened by several minutes by modifications to the software which 1) confine both motion and data taking to those points directly over the cell, regardless of how irregular the shape and 2) concurrent performance of sectional sorting (includes disc I/O)and data taking. The modifications required have been determined and will be implemented in the near future. A reduction in physical scan time of approximately 30% for highly fusiform or dendritic cells is anticipated.

Several utility programs are needed to enable the Chief Operator to control the data acquisition process. The volume of data being collected is large and it is necessary to both protect that previously acquired and provide an easy inventory of cell images on any magnetic tape reel. Currently under development are two short programs to accomplish these tasks. One will perform duplication of all or part of any tape reel. By means of duplication, protection of previously acquired data on any tape reel is achieved. Should a particular operator accidentally destroy the utility of the tape reel to which new cell images are being added, the backup reel can be used to provide another duplicate. This is particularly important when at the end of a tape reel with many images. The potential loss of, say, 600 images, representing many hours of scanning, must be avoided.

The second short program will provide utility listing (inventory) of the contents on any tape reel. All or part of the reel may be listed and some selection of what data is to be listed is permitted. It has been discovered during operations to date that, for various operational reasons, listing of the data on tape is either required or convenient. Further, the particular items for which it is desirable to have printout, frequently vary with the situation. Hence, a program to provide flexible inventory listing of acquired data is being developed.

In order to keep system costs low, pre-existing equipment is heavily relied upon in the development and operation of this system. The scanner

is slow in comparison to other commercially available imaging devices, but fast enough to acquire the data for this project. The resulting data volume is large. It is imperative that, commensurate with the volume of data, its quality also be high. Otherwise, the time and effort taken to acquire the data are wasted. Hence, the strategy employed in this laboratory is, whenever data quality is at stake, time must be taken to assure it. This is the case with software for the protection and enumeration of previously scanned cells.

Relatively minor operational inconveniences in the scanning subsystem arise from time to time. These are corrected on a priority basis as time permits. These technical problems are generally of such nature that scanning is not impeded by their presence, but the process would be either simplified or more conveniently performed by system modification removing the difficulty.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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c. CONTRIBUTING	CARDS			

11. TITLE (Precede with Security Classification Code)

(U) Lesions in Animals Fed Enzyme Inactivated Frozen and Irradiated Meats

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

006500 Food; 014100 Radiobiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
7107 01	CONT.	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING: 76		35
FISCAL YEAR CURRENT: 77		40

19. RESPONSIBLE DOD ORGANIZATION

NAME: Armed Forces Institute of Pathology
Washington, D. C. 20306

ADDRESS:

RESPONSIBLE INDIVIDUAL
NAME: Cowart, E. C. Jr., CAPT MC USN
TELEPHONE: 202-576-2905

21. GENERAL USE

20. PERFORMING ORGANIZATION

NAME: Armed Forces Institute of Pathology
Washington, D. C. 20306

ADDRESS:

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Stedham, M. A., LTC, VC, USA
NAME: Jones, S. R., Lt Col, USAF, VC

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Food, (U) Radiation, (U) Sterilization, (U) Meat, (U) Experimental (U) Laboratory Animals

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To determine the wholesomeness for human consumption of radiation sterilized meat by studying the pathologic effects, if any, of feeding dogs, rats and mice irradiated meat. Preservation of food is vital to military operations.

24. (U) The U. S. Army is conducting contractual studies on the wholesomeness for human consumption of radiation sterilized meat. Pathological results obtained from experimental animals will be statistically analyzed and submitted together with other experimental data to the FDA and USDA to establish a regulation permitting unlimited consumption of radiation sterilized meat. The AFIP serves as monitor and reviewer of the pathologic findings in the contractor's experimental and control animals.

25. (U) 7610-7709. Two site visits to contractors' laboratories, one each to Industrial Bio-Test, Inc., Northbrook, IL and Research 900, Ralston Purina Co., St. Louis, were made during the year. No pathology specimens have been received at the AFIP from the newly initiated chicken study conducted by Research 900 but the necropsy procedures used by their pathologist have been reviewed. All animal feeding studies on the beef study conducted by Industrial Bio-Test have been completed and 1,839 cases have been submitted to the AFIP for review. From a diagnostic standpoint all pathology studies conducted by Bio-Test's pathologists have been satisfactory as there were only minor differences in interpretation from those of the reviewing pathologists at the AFIP. However, some discrepancies have been noted in Bio-Test pathology data resulting from errors in the computer transcription of diagnoses and some variations in the number of tissues available for microscopic study have been noted. Also, blocks were not received by AFIP from Bio-Test on some of the required cases.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

TITLE PAGE

Project No. 1J664713D47

Task No. 00

Title: Lesions in Animals Fed Enzyme Inactivated Frozen and Irradiated Meats

Name and Address of Reporting Installation:

Armed Forces Institute of Pathology
Washington, D. C. 20306

Name of Department: Veterinary Pathology

Period Covered by Report: 1 October 1976 - 30 September 1977

Professional Authors: H. W. Casey, Lt Col, USAF, VC
M. A. Stedham, LTC, VC, USA
S. R. Jones, Lt Col, USAF, VC

Report Control Symbol: RCS-MEDDH-288(R1)

Security Classification: Unclassified

BODY OF REPORT

Project No. 1J664713D847 Title: Lesions in Animals Fed Enzyme Inactivated Frozen and Irradiated Meats

Task No. 00

The need for fresh meat to meet the dietary requirements of large numbers of troops in isolated areas where logistics do not permit the use of refrigeration requires the development of a new preservation and sterilization method for meat products. Preservation by radiation offers a practical means of overcoming these logistical problems while maintaining the wholesome and organoleptic properties of fresh meat.

The AFIP serves as monitor and reviewer for the U. S. Army of pathologic findings in experimental and control animals utilized in the present study now under contract with the Industrial Bio-Test Laboratories, Northbrook, IL, and Research 900, Ralston Purina Company, St. Louis, MO.

All lesions studied to date in animals from the beef study are representative of the spectrum of spontaneous diseases normally expected in a large group of laboratory animals. The leading causes of death from spontaneous diseases in rats have been chronic respiratory disease and progressive renal disease (glomerulonephrosis); however, a wide spectrum of lesions has been observed. Likewise diseases of the respiratory system and urinary tract are most frequently the cause of spontaneous deaths in mice. The pathology studies of the beef project are now centered on the older animals and, as expected, neoplasms are being diagnosed with increasing frequency. Numerous mammary tumors and pituitary adenomas, together with a variety of other neoplasms, have occurred in the rats. The pathology studies in mice to date have been performed on the younger animals, and a limited number of neoplasms have been studied, although several lymphoreticular neoplasms have been diagnosed.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA CB 6608	77 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
76-10-01	D. Change	U	U		NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	6.27.73.A	3E762173A819	00	061
b. CONTRIBUTING	6.27.58.A	3A762758A819		
c. CONTRIBUTING	CARDS 114			

11. TITLE (Precede with Security Classification Code)

(U) Dynamics of Aircraft Accident Victims: Computer Simulation

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

72 07 01

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
	CONT.	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 76		25
FISCAL YEAR CURRENT 77		20

19. RESPONSIBLE DOD ORGANIZATION

NAME: Armed Forces Institute of Pathology

ADDRESS: Washington, D. C. 20306

RESPONSIBLE INDIVIDUAL NAME: Cowart, E. C., Jr., CAPT MC USN

TELEPHONE: 202-576-2905

20. PERFORMING ORGANIZATION

NAME: Armed Forces Institute of Pathology

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS NAME: McMeekin, R. R., M.D. LTC, MC/FS, USA

NAME:

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Computer Simulation, (U) Aircraft Accidents, (U) Occupant kinematics, (U) CALSPAN three dimensional program model

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Computer simulation of the kinematics of occupants in selected aircraft accidents in order to recreate the sequences of injuries and to determine their specific cause(s). This will lead to an increased understanding of the pathogenesis of such injuries and lead to more effective means of preventing them. Examine feasibility of running concurrently the KRASH vehicle impact simulation code so as to enhance the realism of occupant-vehicle interaction.

24. (U) Operate the CALSPAN three-dimensional program as a model using selected Aerospace Pathology Division cases as sources of input data. Correlate the actual injuries observed at autopsy with the injuries predicted from the occupant kinematics of the computer simulation. Use the optimal output features of the CALSPAN code, using subroutines to calculate injury severity indices and time points of maximum injury.

25. (U) 7610-7709. Five additional cases of accidents involving UH1H helicopters have been successfully simulated. The range of decelerative pulses at impact has been extended to include inverted attitudes. Simulations have also been run involving ground impact of individuals involved with out-of-envelope ejection system failures. The body of the report amplifies these considerations.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

TITLE PAGE

Project No. 3A762758A819 Title: Dynamics of Aircraft Accident Victims: Computer Simulation

Task No. 00

Name and Address of Reporting Installation:

Armed Forces Institute of Pathology
Washington, D. C. 20306

Name of Department: Aerospace Pathology Division

Period Covered by Report: 1 October 1976 - 30 September 1977

Professional Authors: J. M. Ballo, LTC, MC, USA
R. R. McMeekin, M.D., LTC, MC/FS, USA

Report Control Symbol: RCS-MEDDH-288(R1)

Security Classifciation: Unclassified

BODY OF REPORT

Project No. 3A762758A819 Title: Dynamics of Aircraft Accident Victims: Computer Simulation

Task No. 00

The previously updated and validated version of the CALSPAN three-dimensional model has been applied to an increasingly complex series of impact situations. The "restart" feature of the program has allowed us to investigate time pulses of between 500 and 1000 msec. without excessive and other integration parameters. Further economies in this regard are expected when the latest received version (Version 17) of the program is implemented. The new integrator of this version will reduce CPU running time by a factor of 60 to 70 percent.

The additional cases subjected to simulation have shown a generally excellent correlation between actual injuries observed and the injury patterns predicted by the simulation. Even in those cases where there is moderate to severe cabin deformation it is apparent that most, if not all, of the lethal injuries are inflicted in the very early stages of the crash sequence. Nevertheless, exact correlation of predicted and occurring injuries will necessitate a routine that incorporates sequential deformation of the program's vehicle panel assemblies. One way to obtain this result is the concurrent operation of a program which would predict the structural deformation of a UH-1H helicopter, or other vehicles, in the crash environment.

During the year there has been continual communication with the U. S. Army Aeromedical Laboratory, Ft. Rucker, Alabama, in an effort to obtain factual data upon which to base simulation attempt.

Accidents involving fatalities caused by ejection seat malfunctions or out-of-envelope escape attempts are another area to which operation of the program has been extended. Investigations have involved varying degrees of man-seat separation, rolling and tumbling after ground strike, interference with seat operation by parachute riser entanglement and rebound of the occupant into the air with subsequent fatal injury on restriking the ground.

Problem areas that have been identified in using the program during the past year include (1) failure of the integration routine during multiple contacts. Prevention of this requires uneconomically short step times. The new integrator in Version 17 will alleviate this shortcoming; (2) simulation runs of 300 and more msec. produce output that is difficult to store and organize. A microfiche system of data output storage, although not entirely satisfactory, will be tested; (3) the output routines developing the pictoral representation of the crash victims are limited to an area of presentation about the inertial origin. Although satisfactory for vehicle simulation, ejection accidents cover a larger physical distance. The relevant "print-plot" routines may be modified to remedy this shortcoming.